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APPLICATION DATA SHEET

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Title of Invention	A METHOD FOR REMOVAL OF AGGREGATE PROTEINS FROM RECOMBINANT SAMPLES USING ION EXCHANGE CHROMATOGRAPHY

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Correspondence address:

Customer Number: 26839

Inventors Information:

Inventor 1:

Applicant Authority Type:	Inventor
Citizenship:	US
Given Name:	Hui
Family Name:	LIU
Residence:	
City of Residence:	Woodlands
State of Residence:	TX
Country of Residence:	US
Address-1 of Mailing Address:	22 South Peaceful Canyon Circle
Address-2 of Mailing Address:	
City of Mailing Address:	Woodlands
State of Mailing Address:	TX
Postal Code of Mailing Address:	77381
Country of Mailing Address:	US
Phone:	
Fax:	
E-mail:	

Inventor 2:

Applicant Authority Type:	Inventor
Citizenship:	US
Given Name:	Ke
Family Name:	TANG

Residence:	
City of Residence:	Houston
State of Residence:	TX
Country of Residence:	US
Address-1 of Mailing Address:	5415 Braesvalley
Address-2 of Mailing Address:	#770
City of Mailing Address:	Houston
State of Mailing Address:	TX
Postal Code of Mailing Address:	77096
Country of Mailing Address:	US
Phone:	
Fax:	
E-mail:	

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Title of Invention	A METHOD FOR REMOVAL OF AGGREGATE PROTEINS FROM RECOMBINANT SAMPLES USING ION EXCHANGE CHROMATOGRAPHY

Application Number :

Date :

First Named Applicant: Hui LIU

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Submitted By:	Elec. Sign.	Sign. Capacity
Cheryl A Liljestrand Registered Number: 45,275	Cheryl A Liljestrand	Attorney

Documents being submitted:	Files
us-request	CaseTNX-1004-usrequ.xml
	us-request.dtd
	us-request.xsl
us-assignment	CaseTNX-1004-usassn.xml
	us-assignment.xsl
	us-assignment.dtd
	Assignment pg1.tif
	Assignment pg2.tif
us-fee-sheet	CaseTNX-1004-usfees.xml
	us-fee-sheet.xsl
	us-fee-sheet.dtd
application-body	Specification-trans.xml
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	mathml2-qname-1.mod
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Description

A METHOD FOR REMOVAL OF AGGREGATE PROTEINS FROM RECOMBINANT SAMPLES USING ION EXCHANGE CHROMATOGRAPHY

Background of Invention

[0001] Protein solutions such as immunoglobulins (IgG), including polyclonal and monoclonal antibodies, routinely contain protein aggregates comprising dimers or higher polymers. In order to administer this solution to a patient, it is necessary to first remove these aggregates to avoid a potentially adverse reaction in the patient.

[0002] The aggregates present in a biological preparation should be controlled below a certain range (e.g. < 1%) in purified antibody preparations for clinical application. Accordingly, it is desirable to provide a process for removing protein aggregates from an antibody preparation by a filtration process which is fast, economic and selectively removes at

least 90% of the aggregates with very little loss of the commercial product.

[0003] Previously, as disclosed in e.g., U.S. Patent No 6,177,548 and US 6,281,336, the typical method to remove the majority of aggregate from an antibody preparation used ion exchange chromatography by a flow-through mode. In this method, the pH and ionic strength were chosen in such a way that a major portion of the contaminants (e.g. aggregates) in the applied solution bound to the anion exchange resin, whereas substantially no antibody monomer was adsorbed to the anion exchange resin, and the desired antibody product was recovered in the flow through.

[0004] As more and more antibodies are developed for therapeutic purposes, it has become necessary to develop a method of quickly determining the pH and salt conditions sufficient to adequately separate aggregates from the antibody of interest. Moreover, many of these aggregates cannot be separated from the antibody monomer of interest by traditional linear gradients, while using a method that is cost efficient and scalable to an industrial manufacturing level. Many resins that can be used at small scales are not useful at large scale because of factors such

as back pressure or cost.

[0005] The present invention provides a new method for quickly determining the conditions necessary and the separation of difficult to remove aggregates from an antibody monomer preparation, wherein the method is scalable to a manufacturing level.

SUMMARY OF INVENTION

[0006] The present invention provides a method for aggregate removal involving a "bind-elute"method for efficient removal of aggregates under conditions suitable for manufacturing scale purification of antibodies. In addition, the antibody product to be purified does not require a preconditioning step, such as tangential flow filtration, as with some previous methods.

[0007] The advantage of the present invention is the ability to purify antibody monomers of interest from an antibody preparation where the aggregates formed are very close in pI value and retention time and the aggregate would not separate from the monomer under normal linear gradient conditions. The present invention provides a method for separation of such an aggregate on a chromatography column such that when the purification process is scaled up to manufacturing levels the aggregate is efficiently re-

moved from the preparation.

[0008] The method involves: (a) choosing an anion-exchange resin that will be of an appropriate size and uniformity for manufacturing level purification of the antibody monomer; (b) Determining the pI of the antibody monomer to be purified; (c) Determine the pH and salt concentration at which the monomer elutes from the column most efficiently by performing a small scale linear gradient at varying pH levels where the pH values chosen are above the pI determined in step (b) using the resin chosen in step (a); and (d) Efficiently removing the aggregate by a step gradient elution.

[0009] Under the selected conditions of the present invention, both the antibody and its aggregate bind to the resin during loading step. Efficient separation of antibody and its aggregate is achieved by applying a step gradient elution program to elute the antibody monomer, leaving the aggregate bound to the anion exchange resin. The present invention achieves a recovery of at least 85% of the desired antibody, preferably 90%, more preferably 95%, with less than 0.1% aggregate remaining.

Brief Description of Drawings

[0010] Figure 1 depicts the antibody concentration and aggregate

levels in each antibody fraction versus elution volume for TNX-901 following anion exchange.

[0011] Figure 2 depicts a chromatogram of the Q-SEPHAROSE® run for TNX-901 at pH 9.2

[0012] Figure 3 depicts an IEF analysis of TNX-901 antibody monomer fraction and aggregate-enriched fraction where Column A is the IEF standard; column B is the aggregate-enriched fraction, and column C is the antibody monomer.

[0013] Figure 4 depicts the antibody concentration and aggregate levels in each antibody fraction versus elution volume for TNX-355 following anion exchange.

[0014] Figure 5 depicts a chromatogram of the Q-SEPHAROSE® run for TNX-355 at pH 8.0.

DETAILED DESCRIPTION

[0015] This invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described herein because they may vary. Further, the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention. As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise, e.g., reference to "an antibody"includes a plu-

rality of such antibodies.

[0016] Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the methods, devices, and materials are described herein.

[0017] All patents and publications mentioned herein are incorporated herein by reference to the extent allowed by law for the purpose of describing and disclosing the proteins, enzymes, vectors, host cells, and methodologies reported therein that might be used with the present invention. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0018] As is well known in the art, ion exchangers may be based on various materials with respect to the matrix as well as to the attached charged groups. For example, the following matrices may be used, in which the materials mentioned may be more or less cross-linked: agarose based (such as SEPHAROSE Fast Flow®(such as Q-SEPHAROSE FF), cellulose based (such as DEAE SEPHACEL®), silica based

and synthetic polymer based, or resins such as such as SuperQ-650 (from TOSOH BIOSEP) and Macro High Q (from BIO-RAD). For the anion exchange resin, the charged groups which are covalently attached to the matrix may, e.g., be diethylaminoethyl (DEAE), quaternary aminoethyl (QAE), and/or quaternary ammonium (Q). In a preferred embodiment of the present process, the anion exchange resin employed is Q-SEPHAROSE FF®, but other anion exchangers can be used.

[0019] Although any of these resins may be used for smale scale purification of antibodies, only those resins of sufficient size and cost are amenable to manufacturing scale separation. If the size of the resin is too small, there is considerable back pressure generated in the system. In addition, the amount of antibody that can be purified is limited.

[0020] Thus, the resin used in the present invention must be of sufficient size to provide efficient scale-up. The resin used in the predetermination step should be the same as that used in the final protocol for manufacturing level purification because one cannot predict the variation in conditions necessary to separate the aggregates if the resin is changed. For purposes of this invention, "manufacturing level" purification is defined as purification using a col-

umn having a bed volume of at least one liter. A particular resin that is useful in smale scale or bench top purification may not be amenable to large scale purification. Such resins include, but are not limited to, Q-SEPHAROSE FF®.

[0021] The appropriate volume of resin used when packing an ion exchange chromatography column is reflected by the dimensions of the column, i.e. the diameter of the column and the height of the resin, and varies depending on, e.g., the amount of antibody in the applied solution and the binding capacity of the resin used.

[0022] Before performing an ion exchange chromatography, the ion exchange resin is preferably equilibrated with a buffer. Any of a variety of buffers are suitable for the equilibration of the ion exchange columns, e.g. sodium acetate, sodium phosphate, tris(hydroxymethyl) amino-methane, Tris, phosphate, bis-Tris, and L-histidine. Persons skilled in the art will appreciate that numerous other buffers may be used for the equilibration as long as the pH and conductivity are about the same as for the applied antibody solution. The elution buffers used in this method may be made of one or more buffer substances to control the pH. The salt used is, e.g., a highly soluble salt, such as sodium chloride or potassium phosphate, but any salt

may be used that maintains the functionality of the antibody and allows elution.

[0023] The elution of the antibody from the anion exchange resin may be performed with a substantially non-denaturating buffer having a pH and ionic strength sufficient to efficiently elute the monomeric antibody, thereby recovering an antibody-containing eluate, while leaving the aggregates bound to the resin. In this context, efficient elution means that at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95% of the antibody loaded onto the anion exchange resin is recovered. Only about 1.0%, preferably only 0.5%, most preferably less than 0.1% aggregates remain in the antibody preparation following anion exchange.

[0024] The elution is advantageously carried out as a step gradient elution step. In the process of the present invention, the preferred buffer used is TRIS/sodium chloride having a pH within the range based on the pI of the antibody.

[0025] It is preferred that the salt concentration of the eluting buffer is sufficiently high to displace the antibody from the resin. However, it is contemplated that an increase in pH and a lower salt concentration can be used to elute the antibody from the resin.

[0026] The present invention allows the effective removal of aggregated antibody from an antibody monomer preparation by Q-SEPHAROSE FAST FLOW® chromatography at pH ranges near, as well as higher than, the pI value of the antibody with varied salt concentrations. After an antibody sample is bound to the resin, the antibody monomer can be eluted by changing the salt concentration or/and pH of the elution solution through a step gradient elution program, leaving the majority of the aggregates bound to the resin. The efficiency of aggregate removal by the optimized step gradient elution is generally 80-90%.

[0027] The antibody to be purified may be of any isotype, including IgG1, IgG2, IgG3, IgG4, IgM, etc.

EXAMPLES

[0028] The following description is presented only to illustrate and describe embodiments of the invention. It is not intended to be exhaustive or to limit the invention to any precise form disclosed. Many modifications and variations are possible in light of the above teaching. For example, although the method as outlined describes in very particular detail the process for monoclonal antibodies TNX901 and TNX355, the method as described would be readily modified as necessary to suit any particular monoclonal

antibody in any given recombinant antibody product.

EXAMPLE 1: SAMPLE PREPARATION PRIOR TO CHROMATOGRAPHY

[0029] A cell culture supernatant containing the desired antibody to be purified may be processed through an immobilized Protein A column, e.g., PROSEP A (Millipore). The antibody sample may also be applied to a hydrophobic interaction column (HIC), e.g., Phenyl SEPHAROSE FAST FLOW® (Amersham Biosciences). The intact antibody fraction binds to the column and then eluted by e.g., a low ionic strength buffer. The antibody preparation may be stored under appropriate conditions at this stage. The present method for aggregate removal may also be performed prior to any purification steps.

[0030] In this example, prior to Q-SEPHAROSE FF® anion exchange chromatography, the monoclonal antibody sample was adjusted to the desired pH for loading the antibody onto a Q-SEPHAROSE FF® column with, e.g., 10 mM Tris in the buffer. Final pH adjustment before each Q-SEPHAROSE FF® run was performed with acidic or basic buffers containing 10 mM Tris. The salt concentration of the sample was adjusted, based upon the designed loading antibody condition. Sample buffer exchange may be done using a variety of column resins, e.g., SEPHADEX G-25 Fine resin,

or by direct conditioning without a column or filtration.

Example 2: Determination of Aggregate Concentration

[0031] The percentage of the aggregates in each prepared sample was determined, including the fractions collected through sample loading, washing and eluting steps. Superdex 200 HR pre-packed analytical column may be used to determine percent aggregate level. Figure 1 depicts the antibody concentration and aggregate levels in each antibody fraction versus elution volume for the antibody TNX-901 following anion exchange.

Example 3: Determination of Antibody Binding and Elution Conditions for TNX-901

[0032] The appropriate salt concentrations used for loading and eluting conditions at the various pH conditions were determined by applying linear gradient of salt concentration increment at the designed pH conditions. The pH range determined for loading the TNX-901 antibody sample was 8.2 to 9.2. From pH 8.2 the antibody and its aggregates can both bind onto Q-SEPHAROSE FF® resin with loading buffer of 10 mM Tris.

[0033] However, the salt concentrations of the buffers for loading and eluting antibody sample may be simultaneously increased when the loading buffer pH is increased from 8.2

to 9.2 for TNX-901 antibody.

[0034] For example, a Q-SEPHAROSE FF® column (1cm ID x 9 cm height) was equilibrated with 10 mM Tris at pH 8.6. The buffer-adjusted antibody sample prepped according to Example 1 was then loaded onto the column. The antibody was loaded at ~17 mg/ml of resin. Under these conditions the antibody and its aggregates bound to the column.

[0035] The column was washed with 5 column volumes of equilibrating buffer (10 mM Tris, pH 8.6) after loading the antibody.

[0036] The bound antibody was eluted with 15 column volumes of the buffer containing 10 mM Tris, pH 8.6 using a linear salt gradient from 0 to 500 mM. Fractions were collected over the course of the elution phase.

[0037] Antibody concentration and aggregate levels were measured in each antibody fraction. The salt concentration of each antibody fraction was also measured by determining its osmolarity. The results are shown in Table 1 (* denotes a value below the detection level of the method).

Table 1: Q-SEPHAROSE Fractionation of TNX-901

Fraction #	Fraction Volume (mL)	Cummulative Elution Volume (mL)	Ab Conc. (mg/mL)	Aggregate (%)	Salt Conc. (mM)

1	7.8	7.8	0.02	0*	65
2	6.5	14.3	0.06	0*	132
3	3.9	18.2	2.89	0.11	180
4	5.0	23.2	17.89	0.77	218
5	8.0	31.2	2.72	0.90	282
6	9.5	40.7	0.11	0*	361

[0038] From these results, it was determined that the NaCl concentration in the first antibody elution fraction was ~65 mM, and thus, the salt concentration that may be used at pH 8.6 to load antibody sample should be below 60 mM. The salt concentration that could be used to elute antibody with a step gradient would be between 65 mM and 135 mM based on the results shown in Table 1 for fractions 1 and 2.

[0039] Figure 1 shows the antibody concentration and aggregate level in each antibody fraction versus eluate volume. Retention time for aggregates is slightly longer than that for the antibody monomer. As one can observe, the differences in retention times for the antibody monomer and aggregates were not sufficient for adequate separation using a linear gradient elution program. Therefore, using the present invention method, the buffers with various pHs were tested with a step-salt gradient, in order to

maximize the separation of TNX-901 antibody monomer from its aggregates. The highest salt concentration for loading antibody sample at each pH condition was determined and the final salt concentration of the elution buffer for eluting antibody monomer at each pH condition was also optimized to achieve the maximal percentage of aggregate removal and the high yield of the antibody recovery.

[0040] Aggregate removal by Q-SEPHAROSE FF® resin in the bind-elute mode was studied over a pH range from 8.2 to 9.2. The amount of antibody loaded was in the range of 15-37 mg/ml of resin, and the amount of aggregate loaded was in the range of 0.1-0.25 mg/ml of resin. Table 2 shows the results of aggregate removal and antibody monomer recovery over this pH range.

Table 2: Loading and Elution Conditions for Aggregate Removal From TNX-901

pH of Loading Sample	8.2	8.4	8.8	8.8	9.2	9.2
Highest Salt Conc. in Sample & Loading Buffers (mM)	0	0	30	30	60	60
Salt Conc. in Elution	20	40	70	80	100	110

Buffer (mM)						
Ab Conc./mL Resin in Load (mg)	16.9	16.3	37.7	15.4	16.9	17.2
Aggregate Conc./mL Resin in Load (mg)	0.11	0.10	0.25	0.09	0.12	0.13
Ab in Flow Thru (%)	6.6	0	13.4	0	0	0
Ab in Eluate (%)	86	93	86	98	89	95
Aggregate Conc. in Load (%)	0.64	0.62	0.66	0.58	0.71	0.77
Aggregate Conc. in Eluate (%)	0.03	0.04	0.14	0.09	n.d.	0.04
Aggregate Removal (%)	95	94	79	84	~100	95

EXAMPLE 4: STEP GRADIENT ELUTION CONDITIONS FOR TNX-901

[0041] Table 3 shows the results for the separation of TNX901 on Q-SEPHAROSE FF® at pH9.2. The Q-SEPHAROSE FF® column was at 1 cm ID x 9 cm bed height. The antibody recovery was over 95% under these tested conditions. The antibody peak pool had about 6 column volumes and contained less than 0.1% aggregates. The chromatogram is shown in Figure 2.

Table 3: Chromatographic Conditions for TNX901

Step	Solution	Flow	Rate	Volume	
		cm/hr	mL/min	CV	mL
Equilibration	10mM TRIS, 60mM NaCl, pH 9.2	122	1.6	8	56
Loading	Sample	122	1.6	4	28
Wash	10mM TRIS, 60mM NaCl, ph 9.2	122	1.6	6	42
Elution	10mM TRIS, 110mM NaCl, pH 9.2	122	1.6	15	105
Strip	1M NaCl	122	1.6	7	49
Regeneration	1M NaCl/1N NaOH	122	1.6	5	35
Storage	0.01N NaOH	122	1.6	5	35

EXAMPLE 5: DETERMINATION OF ANTIBODY BINDING AND ELUTION CONDITIONS FOR TNX-355 ANTIBODY

[0042] The appropriate salt concentrations used for loading and eluting conditions at the various pH conditions were determined by applying linear gradient of salt concentration increment at the designed pH conditions. The pH range determined for loading TNX-355 antibody sample was 6.5 to 8.2. At the antibody loading condition of low conductivity, most of the antibody and its aggregates bind to the

Q-SEPHAROSE FF® resin.

[0043] For example, a Q-SEPHAROSE FF® column (1cm ID x 11 cm height) was equilibrated with 10 mM Tris, pH 8.0, and 25mM NaCl. The buffer-adjusted antibody sample was then loaded onto the column. The buffer-adjusted antibody sample prepped according to Example 1 was then loaded onto the column at ~11 mg/ml of resin. Under these conditions the antibody and its aggregates bound to the column.

[0044] The column was washed with 6 column volumes of equilibrating buffer (10 mM Tris, 25 mM NaCl, pH 8.0) after loading antibody.

[0045] The bound antibody was eluted with 5 column volumes of a linear gradient from 25 mM NaCl to 250 mM NaCl at pH 8.0, and then with 5 column volumes of 250 mM NaCl at pH 8.0. Fractions were collected over the course of the elution phase.

[0046] Antibody concentration and aggregate levels were measured in each antibody fraction. The salt concentration of each antibody fraction was also determined by measuring the osmolarity. The results are shown in Table 4 (* denotes a value below the detection level of the method).

Table 4: Q-SEPHAROSE Fractionation of TNX-3555 at pH8.0

Fraction #	Fraction Volume (mL)	Cummulative Elution Volume (mL)	Ab Conc. (mg/mL)	Aggregate (%)	Salt Conc. (mM)
1	5.7	5.7	0.01	0*	33
2	5.2	10.9	0.29	0*	117
3	5.5	16.4	4.46	0.10	174
4	6.6	23.0	5.76	0.36	239
5	6.8	29.8	2.17	0.62	313
6	5.9	35.7	0.30	0.63	382
7	6.8	42.5	0.09	n.d.	448

[0047] As seen in the results from this table, the salt concentration may be between 33 mM and 117 mM based on the osmolarity of fractions 1-2 and the point where aggregate begins to elute from the column. Figure 4 shows the antibody concentration and aggregate levels in each fraction versus elution volume.

[0048] At pH 8.0, which was 0.8 pH units higher than the upper limit of TNX-355 antibody pI range, both the antibody and its aggregates bound tightly to the Q resin. By gradually increasing the salt concentration in the elution buffer, the antibody monomer, HCP and aggregates were eluted from the Q column at different retention times. A small difference in retention time between the antibody monomer and its aggregates was also observed. An elution step with

the optimized pH and salt concentration, which is close to the second fraction condition, in elution buffer can be used to elute the antibody monomer. The Q-SEPHAROSE FF® columns used for this study were at 1 cm ID with11 - 20 cm bed height.

[0049] Table 5 presents the results under loading and eluting conditions with working pHs at 7.8 and 8.0.

Table 5: Loading and Elution Conditions for Aggregate Removal from TNX-355

pH of Loading Sample	7.8	7.8	8.0	8.0	8.0	8.0	8.0
Run #	1	2	1	2	3	4	5
Column Bed Height (cm)	12	12	11	11	11	20	20
Salt Conc. in Loading buffer (mM)	25	25	25	25	25	25	25
Salt Conc. in Elution Buffer (mM)	75	85	75	85	85	85	90
Ab Conc./mL Resin in Load (mg)	17.8	18.3	9.4	20.3	20.6	20.0	19.7
Aggregate Conc./mL Resin in	0.49	0.49	0.13	0.45	0.71	0.55	0.58

Load (mg)							
Aggregate % in Load	2.78	2.68	1.35	2.22	3.46	2.73	2.96
Aggregate % in Elu-ate	0.26	0.39	<0.05	0.17	0.18	0.15	0.15
Aggregate Removal (%)	91	74	>96	92	95	95	95
Antibody Recover y (%)	90	93	89	96	93	88	89

[0050] The salt concentration in loading buffer was kept at 25 mM sodium chloride with 10 mM Tris for every run. The amount of antibody loaded was in the range of 9-20 mg/ml of resin, and the amount of aggregate was in the range of 0.13-0.58 mg/ml of resin. The antibody recovery was over 88% under these tested conditions. The antibody peak pool had about 6-7 column volumes and contained less than 0.4% of aggregates.

[0051] The results in Table 5 show also that the aggregate removal on Q-SEPHAROSE® resin is more effective at pH 8.0 than 7.8 under the same salt concentration for eluting the antibody. Since the pI range for the TNX-355 antibody is between pH 6.4 and 7.2, using a higher chromatographic pH, that is above the pI range, will result in the better separation of the antibody from its aggregate.

EXAMPLE 6: STEP GRADIENT ELUTION CONDITIONS FOR TNX-355

[0052] TNX-355 antibody aggregate removal by Q-SEPHAROSE FF® resin in the bind-elute mode was studied at a pH above 7.5. The process from an example of the Q-SEPHAROSE FF®run at pH 8.0 is presented in Table 6, and its chromatogram is shown in Figure 5.

Table 6: Chromatographic Conditions for TNX-355

Step	Solution	Flow	Rate	Number Col. Vol.
		cm/hr	mL/min	
Equilibration	10mM TRIS, 25mM NaCl, pH 8.0	122	1.6	8
Loading	Sample	122	1.6	
Wash	10mM TRIS, 25mM NaCl, pH 8.0	122	1.6	5
Elution	10mM TRIS, 85mM NaCl, pH 8.0	122	1.6	9
Strip	10mM TRIS, 250mM NaCl, pH 8.0	122	1.6	5
Regeneration	1M NaCl/1N NaOH	122	1.6	4
Storage	0.01N NaOH	122	1.6	8

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Claims

[c1] A method for the purification of antibody monomers from aggregates for manufacturing scale purification comprising the steps of:
a) choosing an anion exchange resin that can be scaled to a manufacturing level;
b) determining the pH and salt conditions to be used in the manufacturing level purification, wherein the antibody and the aggregates bind to the resin and the antibody is eluted by a step gradient.

[c2] The method according to claim 1, further comprising determining the pI of the antibody monomer to be purified prior to step b.

[c3] The method according to claim 1, wherein a pH and salt concentration for binding aggregates is determined by a linear gradient prior to the step gradient purification.

[c4] The method according to claim 1, wherein the aggregate does not separate sufficiently from the antibody monomer at a manufacturing scale when using a linear salt gradient.

[c5] The method according to claim 1, wherein the anion exchange resin is Q-SEPHAROSE FF
The method according to claim 1, wherein the pH of the binding solution is greater than the pI of the antibody.

[c6] The method according to claim 4, wherein the salt solution comprises NaCl at a concentration ranging from about 0 mM to about 500 mM.

[c7] The method according to claim 5, wherein the NaCl concentration ranges from about 25 mM to about 250 mM.

[c8] The method according to claim 1, wherein the aggregate present in the sample after anion exchange is less than 0.5%.

[c9] The method according to claim 1, wherein the aggregate present in the sample after anion exchange is less than 0.1%.

[c10] The method according to claim 1, wherein about 90% of the antibody is recovered.

[c11] The method according to claim 1, wherein about 95% of the antibody is recovered.

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A METHOD FOR REMOVAL OF AGGREGATE PROTEINS FROM RECOMBINANT SAMPLES USING ION EXCHANGE CHROMATOGRAPHY

Abstract

A method for removing aggregates from antibody preparations comprising a bind-elute method using anion exchange resin and a step gradient wherein the antibody is eluted from the resin. Using this method, at least 90% of the antibody is recovered and only about 1%, preferably less than 0.1% of the aggregate is present following the elution step.

FIGURE 1 Antibody concentration and aggregate level versus elution volume for TNX-901

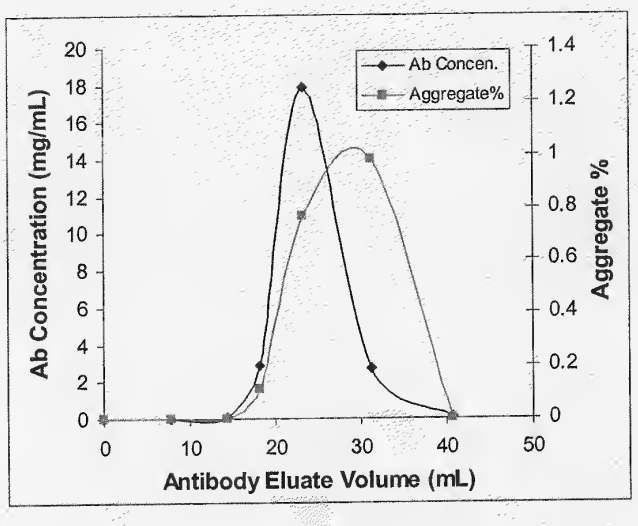
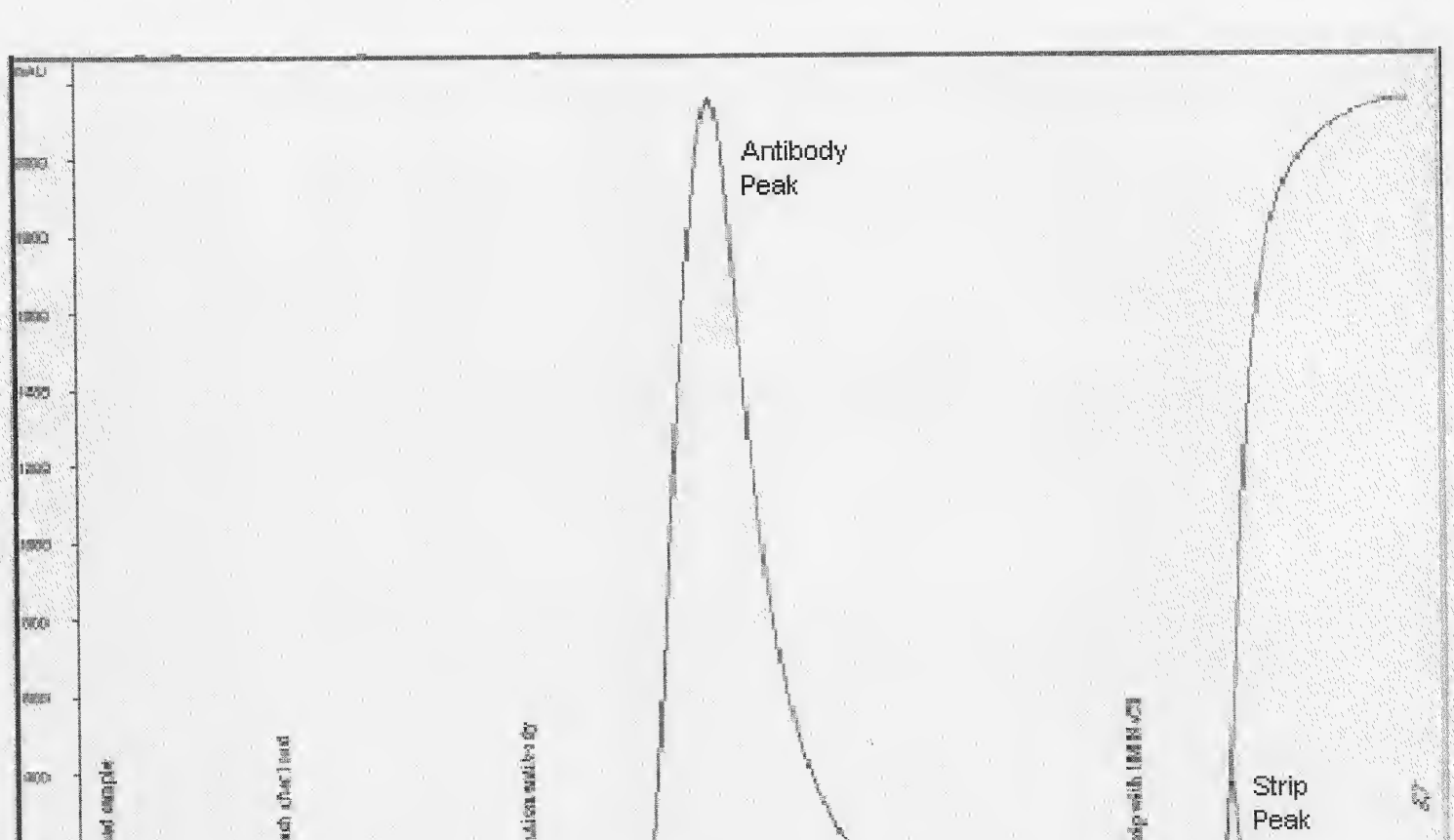


FIGURE 2. Chromatogram of the Q-Sepharose run for TNX-901 at pH 9.2



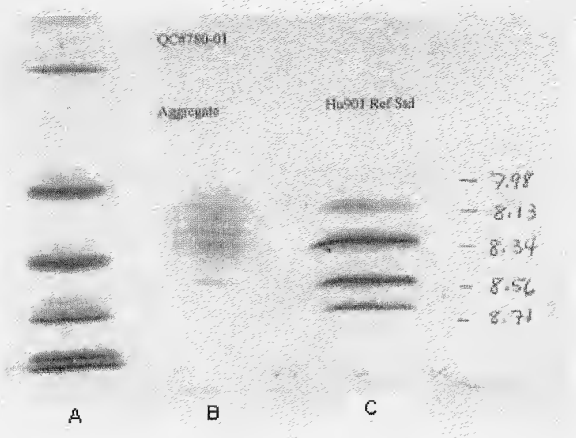


Figure 3. IEF analysis of TNX-901 antibody monomer fraction and aggregate-enriched fraction. Column A: IEF standard; column B: the aggregate-enriched fraction, column C: the antibody monomer.

Figure 4: Antibody concentration and aggregate level versus elution volume for TNX-355

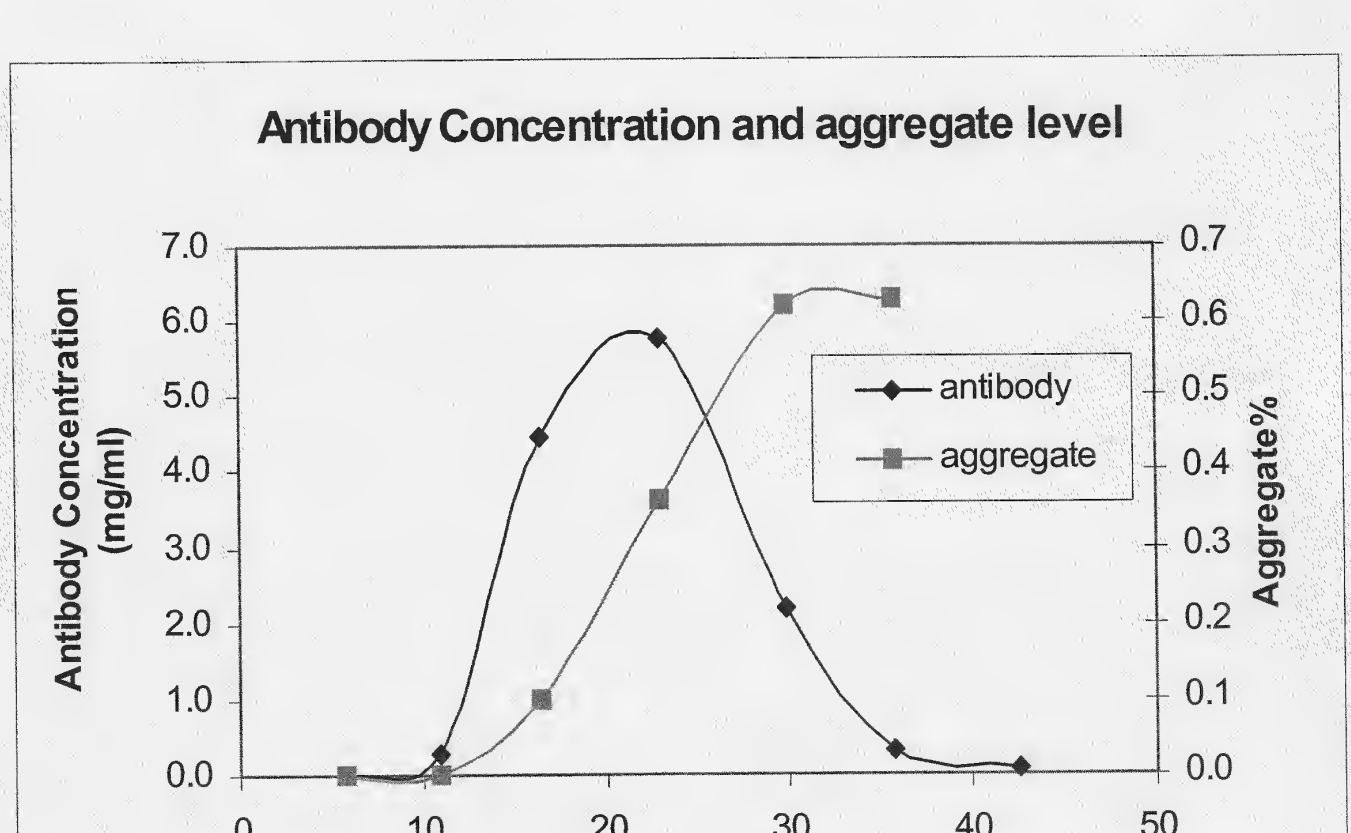


FIGURE 5: Chromatogram of TNX-355 Q-SEPHAROSE FF® run at pH 8.0.